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Insect pathology and fungal endophytes

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ABSTRACT

Fungi that occur inside asymptomatic plant tissues are known as fungal endophytes. Different genera of fungal entomopathogens have been reported as naturally occurring fungal endophytes, and it has been shown that it is possible to inoculate plants with fungal entomopathogens, making them endophytic. Their mode of action against insects appears to be due to antibiosis or feeding deterrence. Research aimed at understanding the fungal ecology of entomopathogenic fungi, and their role as fungal endophytes, could lead to a new paradigm on how to successfully use these organisms in biological control programs.

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1. Introduction

For the last 45 years, insect pathologists have expressed strong optimism concerning the potential for fungal entomopathogens. Reality, nevertheless, indicates that even though there have been clear and significant advances in the field, we still have not overcome the basic obstacles (e.g., moisture constraints, ultraviolet light, formulation) that prevent insect pathogenic fungi from becoming fully commercialized and widely adopted.

Traditionally, insect pathogenic fungi have been studied by two groups of scientists: Entomologists and mycologists. Generally speaking, entomologists have focused on the practical aspects of using a fungal entomopathogen in the field, while mycologists are more interested in fungal taxonomy, mode of action, and phylogenetics. An obvious lacuna caught in the middle is fungal ecology. This knowledge gap has been hinted at in the literature. Cooke (1977a) wrote about "... an almost complete lack of knowledge concerning the behaviour of the fungi under natural conditions" and expanded on this by stating "It is probably that these fungi are normally saprotrophs on dead organic matter but can use the exoskeleton of living insects as an abode when environmental conditions favour this" (Cooke, 1977b). Bruck (2005) elegantly defined the *status quo* about entomopathogenic fungi in two sentences: "There is little or no knowledge of their biology outside of their insect host" and "A completely new approach which shifts the focus of our efforts away from laboratory bioassay data and onto fungal ecology may very well lead to success." De Hoog (1972) in his classic monograph on *Beauveria* and related genera described *Beauveria bassiana* as "...a facultative but highly virulent parasite..." thus implying more than one possible role, although none is mentioned. Have we been overlooking something in our attempts to use fungal entomopathogens as biocontrol agents? Where else might these fungi be found, other than on dead insects? What can we gain from learning more about the ecology of fungal entomopathogens? This paper introduces the reader to the field of fungal entomopathogens as fungal endophytes.

2. Fungal endophytes

One very exciting area in mycology involves the study of fungal endophytes. The term "endophyte" was coined by De Bary (1884) and is used to define fungi or bacteria that occur inside asymptomatic plant tissues. Fungal endophytes are ubiquitous and are dominated by Ascomycota (Arnold and Lutzoni, 2007). The most studied fungal endophytes are grass endophytes in the genus *Neotyphodium* (Clavicipitaceae). These have been shown to have various effects on plant performance and different levels of activity against herbivores (Saikkonen et al., 2006).

3. Fungal entomopathogens as endophytes

Various genera of fungal entomopathogens have been isolated as endophytes in several different plants (Table 1). Some fungal entomopathogens have been reported as naturally occurring endophytes while others have been introduced into the plant using different techniques (Table 1). Studies aimed at experimentally introducing the fungal entomopathogen as an endophyte are aimed at having them act as biological pest control agents against specific pests. Most of these studies have only completed the first stage, i.e., introduction into the plant.

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Table 1
Summary of fungal entomopathogens reported as endophytes in various plants

Fungal species[a]	Plant	Reference
Acremonium spp.	*Coffea arabica* L. (N) (coffee)	Vega et al., in press
Acremonium alternatum	*C. arabica* (N)	Vega et al., in press
Beauveria bassiana	*Zea mays* L. (I) (maize)	Vakili, 1990; Bing and Lewis, 1991, 1992a,b; Jones, 1994; Lomer et al., 1997; Cherry et al., 1999, 2004; Wagner and Lewis, 2000; Lewis et al., 2001
	Z. mays (N)	Jones, 1994; Arnold and Lewis, 2005
	Solanum tuberosum L. (I) (potatoes)	Jones, 1994
	Gossypium hirsutum L. (N, I) (cotton)	Jones, 1994
	Xanthium strumarium L. (I) (common cocklebur)	Jones, 1994
	Datura stramonium L. (N) (jimsonweed)	Jones, 1994
	Lycopersicon esculentum Miller (I) (tomato)	Leckie, 2002; Ownley et al., 2004
	Theobroma gileri Cuatrec. (N)	Evans et al., 2003
	Carpinus caroliniana Walter (N) (ironwood)	Bills and Polishook, 1991
	Pinus monticola D. Don (N) (western white pine)	Ganley and Newcombe, 2005
	Papaver somniferum L. (I) (opium poppy)	Quesada-Moraga et al., 2006
	Phoenix dactylifera L. (I) (date palm)	Gómez-Vidal et al., 2006
	Musa paradisiaca L. (I) (banana)	Akello et al., 2007
	C. arabica (N, I)	Posada and Vega, 2006; Posada et al., 2007; Vega et al., in press, submitted
	Theobroma cacao L. (I) (cocoa)	Posada and Vega, 2005
Beauveria brongniartii	*C. arabica* (N)	Vega et al., submitted
Cladosporium spp.	*C. arabica* (N)	Vega et al., in press
	Avicennia officinalis L., *Rhizophora mucronata* Lam., *Sonneratia caseolaris* (L.) Engl. (N) (mangroves)	Ananda and Sridhar, 2002
Clonostachys rosea	*C. arabica* (N)	Vega et al., in press
Isaria spp.	*C. arabica* (N)	Vega et al., in press
Lecanicillium dimorphum & *L. c.f. psalliotae*	*P. dactylifera* L. (I)	Gómez-Vidal et al., 2006
Paecilomyces spp.	*Musa acuminata* Colla (N) (banana)	Cao et al., 2002
	Oryza sativa L. (N) (rice)	Tian et al., 2004
Paecilomyces farinosus (= *Isaria farinosa*)	*C. caroliniana* (N)	Bills and Polishook, 1991
Verticillium (= *Lecanicillium*) *lecanii*	Araceae	Petrini, 1981
	C. caroliniana (N)	Bills and Polishook, 1991
	Arctostaphylos uva-ursi (L.) Sprengel	Widler and Müller, 1984

An "N" or "I" following the scientific name for the plant indicates that the fungal species was reported as a naturally occurring endophyte (N) in contrast to those that were introduced into the plant using different techniques (I). Plant common name is given in parenthesis when applicable.
[a] All Ascomycota: Hypocreales, except for *Cladosporium* (Ascomycota: Capnodiales).

Based on studies where insect performance on plants containing endophytic fungal entomopathogens has been examined, it can be surmised that fungal presence in plant tissues causes feeding deterrence or antibiosis, likely due to production of metabolites (see Vega et al., in press). This is supported by reduced tunneling by *Ostrinia nubilalis* in maize plants with endophytic *B. bassiana* and no symptoms of mycosis (Lewis and Bing, 1991; Bing and Lewis, 1991, 1992a,b, 1993). Cherry et al. (1999, 2004) reported similar results with maize stem borers (*Sesamia calamistis*). Recently, Powell et al. (2007) reported mycosis in *Helicoverpa zea* larvae feeding on tomato plants with endophytic *B. bassiana*, which would indicate the presence of conidia as infective propagules or *per os* infection. Electron microscopy did not detect *B. bassiana* conidia inside maize plants known to contain the fungus as an endophyte (Wagner and Lewis, 2000); therefore, it will be interesting to learn more about mechanisms for *H. zea* mycosis seen in tomato plants with endophytic *B. bassiana*.

Vega et al. (in press) isolated several genera of fungal entomopathogens, including *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys*, and *Isaria* (Table 1) as fungal endophytes in coffee plants collected in Colombia, Hawaii, and Puerto Rico. Two isolates, *B. bassiana* and *Clonostachys rosea*, were tested in laboratory bioassays and shown to be pathogenic to the coffee berry borer, *Hypothenemus hampei* (Ferrari).

4. Summary

In a survey of fungal endophytes in coffee plants, a total of 843 fungal isolates were recovered and sequenced, yielding 257 unique ITS genotypes (Vega et al., submitted for publication). This enormous fungal endophyte diversity within one plant species points at a niche for fungi in general, and for entomopathogenic fungi in particular. Why are these fungal endophytes present in the plant? Vega et al. (submitted for publication) have posited the possibility that some of these could be "influential passengers," having some effect on plant performance, while others might simply be "accidental tourists," with no role on the plant. Cascading effects of endophytes could also be possible. For example, in addition to the direct negative effects of grass fungal endophytes on nematodes (West et al., 1988; Kimmons et al., 1990), it has been found that in some instances, they protect herbivores against entomopathogenic nematodes (Kunkel and Grewal, 2003; Kunkel et al., 2004).

Insect pathologists have traditionally seen entomopathogenic fungi as having only one role, i.e., killing an insect pest. Other roles are also possible. For example, Ownley et al. (2004, 2008) have shown that *B. bassiana* plays a role against plant pathogenic fungi. St. Leger (2008) and Bruck (2005) have demonstrated a strong association between *Metarhizium anisopliae* and the rhizosphere.

As an immediate step in advancing the field, it would be advisable to sample more agricultural crops for the presence of entomopathogenic fungi as endophytes. This could be coupled with studies aimed at determining whether manipulated inoculation and subsequent establishment of entomopathogenic fungi is possible. In addition, information on *in planta* distribution of entomopathogenic fungi is needed to determine if fungal presence is systemic or localized, and whether there is vertical transmission via the seeds.

Finally, it is imperative to learn more about the fungal ecology of entomopathogenic fungi in order to understand what other roles they might play in nature. This approach should lead to innovative research initiatives that will enhance our understanding of these organisms and likely result in a new paradigm for biological control using fungal entomopathogens.

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